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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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INTELLECTUAL PROPERTY / TECHNOLOGY LAW
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EXAMINER	
DAVIS, MINH TAM B	

ART UNIT	PAPER NUMBER
1642	15

DATE MAILED: 01/30/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/878,454	MONTEIRO ET AL.	
	Examiner	Art Unit	
	MINH-TAM DAVIS	1642	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *25 October 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-24* is/are pending in the application.

4a) Of the above claim(s) *5-7 and 10-22* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-4,8,9,23 and 24* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *10* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Applicant's election with traverse of claims 1-11, 23, mutation in the amino acid residue at position 116-128 in the EF-N calcium binding hand of SEQ ID NO:2 in Paper No. 14 is acknowledged. The traversal is on the ground(s) that 1) Claims 12 and 17 should be examined together because it is of great economy for the Office and for Applicant, and because they are drawn to closed subject matters, 2) The product and process claims are interdependent and should be examined together. Applicant further argues that the court has recognized that it is in the public interest to permit Applicant to claim several aspect of their invention together in one application. Applicant recites *Studiengesellschaft Kohle mbH v. Northern Petrochemical Co. and Gerber Garment Technology Inc. v. Lectra Systems Inc.*, and argues that restriction requirement with inadequate authority can lead to situations in which applicant's patent rights are exposed to uncertainty and even extinguished, because patents issuing on divisional applications that are filed to prosecute claims that the Examiner held to be independent and distinct can be vulnerable to legal challenging alleging double patenting and are not protected from US 35 121, and because it is far from clear that the step of filing terminal disclaimer after the issuance of patent on the divisional application is available.

The recitation of *Studiengesellschaft Kohle mbH v. Northern Petrochemical Co. and Gerber Garment Technology Inc. v. Lectra Systems Inc.* is acknowledged.

Applicant arguments have been considered but are not found persuasive for the following reasons: 1) Claims 12 and 17 are drawn to two different mutants of SEQ ID NO:2, having different structure and properties, 2) The products and processes are

distinct for reasons already set forth in previous Office action, and further, the scope of the process claims is different from the scope of the product claims, and 3) It is proper to restrict the claims because they are drawn to inventions that are either independent or distinct for reasons already set forth in previous Office action (see MPEP 803, 806.04-806.04(i) and 806.05-806.05(i)). It is further noted that due to the presence of the linking claims, unless the original linking claims are allowable, the different cited inventions set forth in the previous Office action of 09/25/2202 are groups and not species (see MPEP 804.01).

The requirement is still deemed proper and is therefore made FINAL.

Applicant adds new claim 24 which is related to claims 1-4, 8-9 and 23 and is not new matter.

Accordingly, claims 1-4, 8-9, 23-24 are examined, wherein the claims are only examined to the extent of a method for reducing induced apoptosis mediated by protein-protein interaction, or a method for reducing apoptosis, comprising administering a mutant of SEQ ID NO:2, wherein the mutation is a substitution at at least one amino acid residue in the calcium-binding hand EF-N at positions 116 to 128 of SEQ ID NO:2. Claims 5-7, 10-11 are withdrawn from consideration as being drawn to non-elected invention.

OBJECTION

1 Claims 8, 23 and 24 are objected to because part of claims 8, 23 and 24 are drawn to non-elected invention.

2. Claims 1, 23, 24 are objected to because it is not clear protein-protein interaction refers to the interaction between which proteins.

SEQUENCE RULE COMPLIANCE

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 C.F.R. 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 C.F.R. 1.821-25 for the following reasons:

The figure legends of figures 1, 4 and the sequences in the specification, e.g. on page 27, are not accompanied by sequence identification numbers.

Claim Rejections - 35 USC § 112, SECOND PARAGRAPH

Claims 1-4, 8-9, 24 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

1. Claims 1-4, 8-9, 24 are indefinite, because claims 1, 24 are missing a step of administrating a mutant calcium binding protein.

2. Claim 9 is indefinite, because it is not clear what "amine counterpart" means.

Claim Rejections - 35 USC § 112, FIRST PARAGRAPH, SCOPE

Claims 1-4, 8-9, 23-24 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for reducing apoptosis *in*

vitro, does not reasonably provide enablement for a method for inducing apoptosis *in vivo*. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claims 1-4, 8-9, 24 are drawn to a method to reduce induced apoptosis mediated by protein-protein interaction, comprising inhibiting interaction of presenilin 2 of SEQ ID NO:1 with a calcium-binding protein comprising SEQ ID NO:2, which inhibiting is facilitated or effected by a substitution of at least one amino acid residue in the calcium-binding EF hand including amino acid residues at positions 116 to 128 of SEQ ID NO:2, or by substitution of at least one acidic residue in the EF-hand of SEQ ID NO:2 with its amine counterpart. The calcium-binding protein has reduced interaction with presenilin 1 of SEQ ID NO:3 relativve to the interaction with presenilin 2. Claim 23 is drawn to a method for reducing apoptosis in neuronal cells, comprising administering a calcium-binding protein comprising SEQ ID NO:2, wherein the calcium binding protein comprises at least one substitution in the amino acid residues in the calcium-binding EF hands.

It is noted that the claims as broadly written, encompass both *in vitro* and *in vivo* method for reducing apoptosis.

The specification discloses that mutation in the genes presenilin 1 (PS1) and presenilin 2 (PS2), is associated with early onset of familial Alzheimer's disease (AD), wherein missense mutations are generally found in residues that are conserved between these two proteins, with rare exceptions of in-frame splice deletion and

premature truncations (p.2 last paragraph, bridging p.3). The specification further discloses that mechanisms by which mutations of PS1 and PS2 cause AD is not known, and that PS1 and PS2 genes are both ubiquitously expressed, but at low protein levels (p. 3, second paragraph). The specification also discloses that endogenous calmyrin, i.e. a calcium-binding protein of SEQ ID NO:2, has faint staining in primary and established cell cultures (p. 29, lines 1-2). In other words, SEQ ID NO:2 is expressed at low protein levels in primary and established cell cultures.

The specification discloses that in Hela cells transfected with presenilin 2 (PS2) comprising SEQ ID NO:1 together with a calcium-binding protein or calmyrin comprising SEQ ID NO:2, SEQ ID NO:1 binds to SEQ ID NO:2, as shown by yeast two hybrid system, using the PS2 loop B as a trap (p.23), and by colocalization in the ER of the overexpressed SEQ ID NO:2 in the presence of the overexpressed SP2 of SEQ ID NO:1, and coimmunoprecipitation of the two proteins (p.28-30). The transfection of both SEQ ID NO;1 and SEQ ID NO:2 induces synergistically an apoptosis level higher than the level of apoptosis induced by either SEQ ID NO:1 or SEQ ID NO:2 alone (p.31, last paragraph). The domain of the calcium-binding protein comprising SEQ ID NO:2 necessary for its binding to the PS2 of SEQ ID NO:1 however is not disclosed.

The specification further discloses that preventing myristoylation of SEQ ID NO:2 reduces its half life by half, and that myristic acid on SEQ ID NO:2 is essential for its targeting to ER, where it is localized with PS2 of SEQ ID NO:1 (p.38, second and third paragraph).

In addition, the specification discloses substitution of Aspartic acid 127 of SEQ ID NO:2 to Asparagines 127 in the calcium-binding EF-N hand of SEQ ID NO:2 (EF-N mutant) (p.37, first paragraph), which comprises amino acids 116 to 128 of SEQ ID NO:2 (figure 4). The specification discloses that coexpression of the EF-N mutant with PS2 of SEQ ID NO:1 decreases the cell death level below the level seen with PS2 alone (p.39, and figure 40).

Moreover, the specification discloses that while disrupting the myristoylation site of SEQ ID NO:2 prevents the protein from altering Calcium regulation, mutation the Calcium binding affinity produces a wild type like functional protein (p. 44, lines 24-26).

Furthermore, the specification discloses that SEQ ID NO:2 preferentially interact with PS2 of SEQ ID NO:1 as compared to PS1 of SEQ ID NO:3 (p.24, first two paragraphs).

One cannot extrapolate the teaching of the specification to the scope of the claims because of the following reasons:

1) It is not clear which disease conditions are related to the induced apoptosis mediated by the protein-protein interaction between PS2 of SEQ ID NO:1 and the calcium binding protein of SEQ ID NO:2.

Although familial AD is correlated to mutation of PS1 and PS2, such as missense mutations, in-frame splice deletion and premature truncations, there is no correlation between familial AD and induced apoptosis caused by the interaction between PS2 of SEQ ID NO:1 and the calcium binding protein of SEQ ID NO:2.

Further, there is no indication that apoptosis in neuronal cells *in vivo* are due to the protein-protein interaction between PS2 of SEQ ID NO:1 and the calcium binding protein of SEQ ID NO:2 or by the overexpression of PS2, because the induced apoptosis has been shown only from the transfection and overexpression of PS2 of SEQ ID NO:1 and SEQ ID NO:2 in Hela cells in culture, which are not the same as neural cells *in vivo*.

2) Further, it is unpredictable that *in vivo*, there is induced apoptosis caused by the protein-protein interaction between PS2 of SEQ ID NO:1 and the calcium binding protein of SEQ ID NO:2, and that said apoptosis could be reduced by administering SEQ ID NO:2 which is mutated at at least one amino acid from the amino acid positions 116-128, because *in vitro* conditions of Hela cells in culture transfected with SEQ ID Nos: 1 and 2 cannot represent the complex *in vivo* conditions. In transfected cells, the transfected genes are overexpressed, and the over-abundance of the expressed proteins could artificially cause interaction between the overexpressed proteins. This is not the case of *in vivo* conditions, wherein PS1, PS2 and SEQ ID NO:2 are expressed at low protein levels, as disclosed in the specification (p. 3, second paragraph, and p.29, lines 1-2). Moreover, it is well known in the art that how a cell responds to an apoptotic signal depends on the intracellular concentrations of particular family members of proteins that are related to apoptosis (Oltvai et al, 1994). Thus it is unpredictable that overexpression of SEQ ID NOs:1 and 2 would not artificially change the apoptotic response of the transfected cells.

Further, characteristics of cultured cell lines generally differ significantly from the characteristics of a primary tumor. Drexler et al (Leukemia and Lymphoma, 1993, 9:1-25) specifically teach, in the study of Hodgkin and Reed-Sternberg cancer cells in culture, that the acquisition or loss of certain properties during adaptation to culture systems cannot be excluded and that only a few cell lines containing cells that resemble the *in-vivo* cancer cells have been established and even for the *bona fide* cancer cell lines it is difficult to prove that the immortalized cells originated from a specific cancer cell (see attached abstract). Moreover, Embleton et al (Immunol Ser, 1984, 23:181-207) specifically teaches that in procedures for the diagnosis of osteogenic sarcoma, caution must be used when interpreting results obtained with monoclonal antibodies that had been raised to cultured cell lines and specifically teach that cultured tumor cells may not be antigenically typical of the tumor cell population from which they were derived and it is well established that new artifactural antigens can occur as a result of culture (see attached abstract). Hsu (in Tissue Culture Methods and Applications, Kruse and Patterson, Eds, 1973, Academic Press, NY, see abstract, p.764) specifically teaches that it is well known that cell cultures *in vitro* frequently change their chromosomal constitutions (see abstract). The evidence presented clearly demonstrates that in cell culture systems, in general, and in cancer derived cell lines in particular, that artifactural chromosome constitutions and antigen expression are expected and must be taken into account when interpreting data received from cell line assays. Further, Freshney (Culture of Animal Cells, A Manual of Basic Technique, Alan R. Liss, Inc., 1983, New York, p4) teach that it is recognized in the art that there are

many differences between cultured cells and their counterparts *in vivo*. These differences stem from the dissociation of cells from a three-dimensional geometry and their propagation on a two-dimensional substrate. Specific cell interactions characteristic of histology of the tissue are lost. The culture environment lacks the input of the nervous and endocrine systems involved in homeostatic regulation *in vivo*. Without this control, cellular metabolism may be more constant *in vitro* but may not be truly representative of the tissue from which the cells were derived. This has often led to tissue culture being regarded in a rather skeptical light (p. 4, see Major Differences *In Vitro*). Further, Dermer (Bio/Technology, 1994, 12:320) teaches that, petri dish cancer is a poor representation of malignancy, with characteristics profoundly different from the human disease Further, Dermer teaches that when a normal or malignant body cell adapts to immortal life in culture, it takes an evolutionary -type step that enables the new line to thrive in its artificial environment. This step transforms a cell from one that is stable and differentiated to one that is not, yet normal or malignant cells *in vivo* are not like that. The reference states that evidence of the contradictions between life on the bottom of a lab dish and in the body has been in the scientific literature for more than 30 years. Clearly it is well known in the art that cells in culture exhibit characteristics different from those *in vivo* and cannot duplicate the complex conditions of the *in vivo* environment involved in homeostasis and cell-cell interations. Thus, based on the cell culture data presented in the specification, it could not be predicted that, in the *in vivo* environment, there is induced apoptosis caused by the protein-protein interaction between PS2 of SEQ ID NO:1 and the calcium binding protein of SEQ ID NO:2, and

that said apoptosis could be reduced by administering SEQ ID NO:2 which is mutated at at least one amino acid from the amino acid positions 116-128.

3) In addition, there is no teaching of how the mutated SEQ ID NO:2 could be targeted to and enter through the membrane barrier of the target cells. A therapeutic agent such as the claimed mutated SEQ ID NO:2 (EF-N mutant) must accomplish several tasks to be effective. It must be delivered into the circulation that supplies the target cells and interact at the proper site of action and must do so at a sufficient concentration and for a sufficient period of time. *In vitro* assays cannot duplicate the complex conditions of *in vivo* therapy. In the assays, the mutated SEQ ID NO:2 is over-expressed together with SEQ ID N:1 inside the transfected Hela cells. This is not the case *in vivo*, where exposure to the target site may be delayed or inadequate. In addition variables such as biological stability, half-life or clearance from the blood are important parameters in achieving successful therapy. As written, the mutated SEQ ID NO:2 is not myristoylated, and thus is not stable, as disclosed in the specification (p.38, second paragraph) and would not be targeted to the endoplasmic reticulum, where it is found to colocalize with and presumably to interact with PS2 (specification, p. 38, last paragraph). Further, the mutated polypeptide may be inactivated *in vivo* before producing a sufficient effect, for example, by proteolytic degradation, immunological activation or due to an inherently short half life of the protein and the *in vitro* tests of record do not sufficiently duplicate the conditions which occur *in vivo*. In addition, the polypeptide may not otherwise reach the targert because of its inability to penetrate tissues or cells where its activity is to be exerted, may be absorbed by fluids, cells and

tissues where the polypeptide has no effect, circulation into the target area may be insufficient to carry the polypeptide and a large enough local concentration may not be established.

4) Further, it is unpredictable that administration of the mutated SEQ ID NO:2 (EF-N mutant) would facilitate or effect the inhibition of the interaction between PS2 of SEQ ID NO:1 and the calcium binding protein of SEQ ID NO:2.

Although coexpression of the EF-N mutant of SEQ ID NO:2, which has mutation of Aspartic acid 127 to Asparagine 127 in the calcium-binding EF-N hand of SEQ ID NO:2, together with PS2 of SEQ ID NO:1 decreases the cell death level below the level seen with PS2 alone, the mechanism of said decrease in cell death level is not known. Thus, it is unpredictable that said decrease in apoptosis is due to inhibiting the interaction between PS2 of SEQ ID NO:1 and SEQ ID NO:2, in view of the fact that 1) the mechanism of said decrease in cell death level is not known, 2) the mutation is in the calcium binding region of SEQ ID NO:2, which cannot be predictably to be in the region of SEQ ID NO:2 necessary for the binding and interaction with PS2.

5) In addition, it is unpredictable that "any substitution at any amino acid residue", at positions 116 to 128 of SEQ ID NO:2, other than substitution of Aspartic acid 127 with Asparagine127 would reduce apoptosis.

The claims 8, 23, 24, as written, encompass a method for *in vivo* reducing apoptosis, comprising administering a calcium-binding protein comprising SEQ ID NO:2, wherein "any amino acid residue" in the EF-hand comprising amino acid residues at positions 116 to 128 of SEQ ID NO:2 is substituted with any other amino acid.

It is noted that the mutation from Aspartic acid 127 to Asparagine 127 in the calcium binding region of the calcium binding protein SEQ ID NO:2 is a conservative substitution, and that said mutation does not effect the regulation of Calcium, as disclosed in the specification (p.44, lines 25-26. Further, in view of the fact that 1) one cannot predict that the calcium binding region of SEQ ID NO:2 is involved in the binding and interaction with SP2, and 2) the mechanism by which the mutated SEQ ID NO:2 (EF-N mutant) reduces the apoptosis induced by SP2 is not known, one cannot reasonably predict that substitution of any amino acid in the calcium binding region comprising amino acids 116-128 of SEQ ID NO:2 would have any effect on reducing apoptosis.

Further, the scope of the claims includes a method for reducing apoptosis using numerous structural variants. Applicants have not shown how to make and use the claimed variants which are capable of functioning as that which is being disclosed.

Protein chemistry is probably one of the most unpredictable areas of biotechnology. For example, replacement of a single lysine residue at position 118 of acidic fibroblast growth factor by glutamic acid led to the substantial loss of heparin binding, receptor binding and biological activity of the protein (Burgess et al. Journal of Cell Biology, 1990, 11: 2129-2138). In transforming growth factor alpha, replacement of aspartic acid at position 47 with alanine or asparagine did not affect biological activity while replacement with serine or glutamic acid sharply reduced the biological activity of the mitogen (Lazar et al. Molecular and Cell Biology, 1988, 8: 1247-1252). Similarly, it has been shown that aglycosylation of antibodies reduces the resistance of the

antibodies to proteolytic degradation, while CH2 deletions increase the binding affinity of the antibodies (see Tao. et al. The Journal of Immunology, 1989, 143(8): 2595-2601, and Gillies et al. Human Antibodies and Hybridomas, 1990, 1(1): 47-54). These references demonstrate that even a single amino acid substitution or what appears to be an inconsequential chemical modification will often dramatically affect the biological activity and characteristic of a protein.

In view of the above, it would have been undue experimentation for one of skill in the art to practice the claimed invention as broadly as claimed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MINH-TAM DAVIS whose telephone number is 703-305-2008. The examiner can normally be reached on 9:30AM-4:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, ANTHONY CAPUTA can be reached on 703-308-3995. The fax phone numbers for the organization where this application or proceeding is assigned are 703-872-9306 for regular communications and 703-872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-0916.

MINH TAM DAVIS

January 25, 2003

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